An illustrative method for demonstration of denaturation-renaturation of subunits of collagen

E. KULONEN AND T. HOLLMÉN
Department of Medical Chemistry, University of Turku, Turku, Finland

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THE PURPOSE OF THIS PAPER is to demonstrate a combination of temperature gradient with the electrophoresis on starch gel sheets. In the present conditions the denatured subunits of collagen migrate but the native and renatured molecules remain on the starting line. The linear temperature gradient, usually perpendicular to the current, was created by thermal conduction in an aluminum block between the opposite edges, which are maintained at two different, constant temperatures.

EXPERIMENTAL METHODS

The apparatus is described separately (1), as are also the electrophoretic conditions (2). The dimensions of the starch-gel sheet were 110 x 110 x 6 mm, and it was connected with the electrode vessels by the extensions of the gel. The whole apparatus was insulated from the ambient temperature with a layer of plastic foam. For the electrical insulation between the gel and the aluminum block, the starch gel was covered with two membranes of polythene.

Acetic acid-soluble, rat tail tendon collagen was used as material, dissolved in the electrophoresis buffer (acetate buffer, $\mu = 0.017$, pH 4.7) to the concentration of 8 mg/ml. The concentration of hydrolyzed starch gel was 14.7 g/100 ml (w/v).

The sample was imbibed into a 5 x 109 mm strip of Whatman no. 3MM chromatographic paper, which was then inserted into a slot made across the whole width of the gel sheet, approximately 3 cm from the anodic margin. The sample was applied in the cold room into a cold gel when the denaturation of native collagen was studied and at +37 C when the reconstitution of denatured collagen was to be followed. The gel was then covered with polythene membranes and the gradient block, which had already been stabilized to the temperature gradient, was pressed against the gel. In some experiments the gel had already been stabilized to the gradient under the block before the application of the sample.

The voltage was selected so that the production of ohmic heat in the gel never exceeded 1 % of the flow of thermal energy in the aluminum block.

RESULTS

In the studies on the renaturation of denatured collagen (Fig. 1, lower section) it appeared that each subunit reconstituted at a characteristic temperature-dependent rate, which has been shown with the classical methods for pure subunits by Piez and Carrillo (3). The collagen sample was denatured for 30 min at +40 C and allowed to reconstitute for 60 min in the gradient before the connection of the current. The electrophoretic run was carried out at 80 v/25 ma for 6 hr. The reconstituted molecules remain on the starting line. They can be identified by a second run at such temperature, where they are unfolded. The gelatin molecules, which have been driven electrophoretically into the gel, do not fold there any longer. The migration rates of unfolded molecules did not change when they entered the gel segments below their transition temperatures. The reconstitution as tested by this method seems to be an all-or-nothing process, because the fading ends are not curved at all and there is no tailing or blurring of the bands.

In the studies on the denaturation of native collagen (Fig. 1, upper section) this method gives the final equilibrium state after a long denaturation time. The sample of soluble collagen was inserted in the gel which had been previously equilibrated to the temperature gradient and the gradient maintained for 30 min before the first electrophoretic run at 60 v/23 ma for 8 hr. During the second run the temperature in the whole gel was +45 C. All the subunits are liberated at the same temperature (in contrast to the pattern at reconstitution). The effect of higher temperatures is also shown in Fig. 1 (to the right). The progressive disappearance of other units except that related to $\alpha 1$ is to be noted. The sample of soluble collagen was denatured for 30 min in a gradient of comparatively high temperatures before the run at 40 v/10 ma for 19 hr in the same temperature gradient.

DISCUSSION

The present criterion of denaturation, e.g., the appearance of subunits, differs from those observed in the studies employing the classical methods, and the transi-

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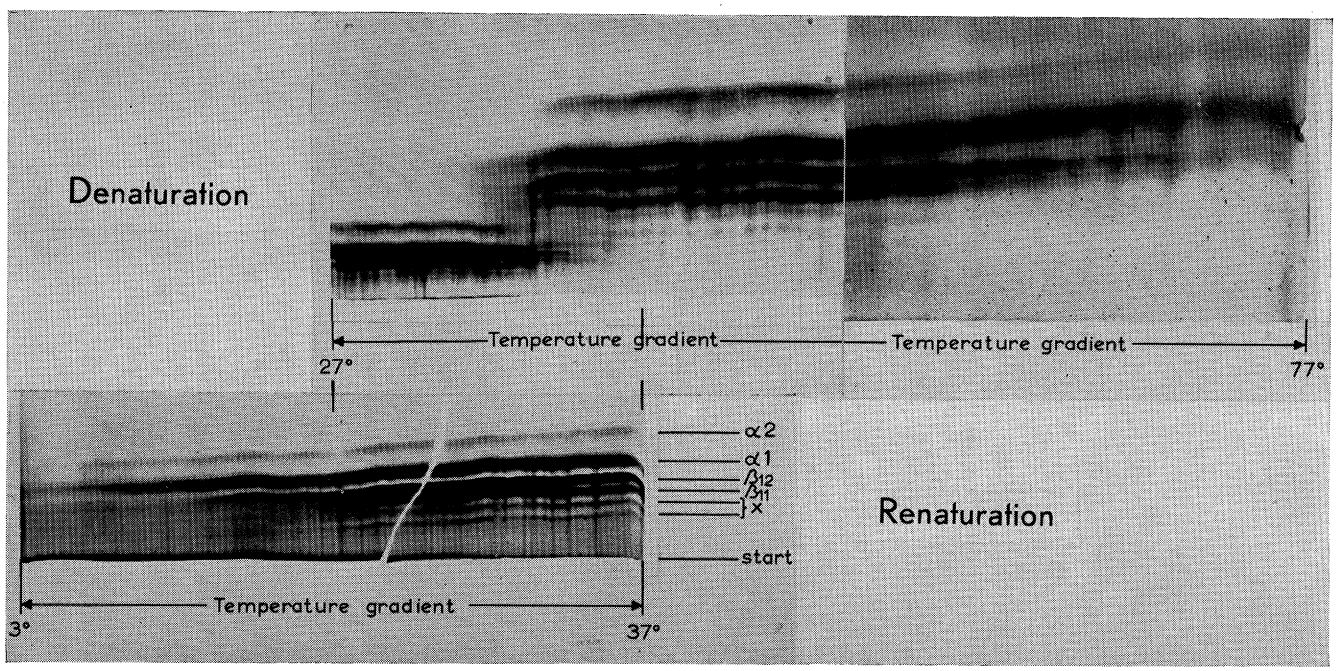

FIG. 1. Combination of three starch-gel electrophoretic patterns at various gradients for the comparison of denaturation (in the temperature range +27 to +77 C) and renaturation.

tion temperatures should be compared with some caution.

The present procedure is simple, easy, and fast. Every component in a mixture is analyzed separately without any preceding fractionation process. The band pattern is conspicuous and, because of its continuity through the temperature range, it is easy to assign the bands in spite of eventual differences in the mobility. On the other hand, this method is less accurate than the classical methods, the presence of starch in the medium may affect the properties of the materials to be studied, and the choice of the ionic environment is restricted.

The results of the reconstitution experiment confirm that the collagen fold can be formed in various combinations ot the subunits (K. Kühn, C. Tkocz, B. Zimmerman, and G. Beier, personal communication). Perhaps these differences between the subunits could be exploited for preparative purposes.

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